Project No.
80 Book No.
TITLE 23 mer degradation: V, OV, Tne buffers: Cheng vs. Vent vs. Klenteg

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	①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩	⑪
Cheng buffer 5X	20 →										
10X Klenteg buffer *				10 →							
Vent buffer							10 —				
Tag storage buffer				2	2 —						
Mg OAc 12 mM	9.5 →										
Mg SO4 100 mM				1.2 µl →							
glycerol 50%										16 —	
DMSO 100%											
32P 23mer **	3 µl —										
Vent pol 0.05 u/µl	2			2			2			2	
Deep Vent 0.05 u/µl		2			2			2			2
Tne 0.05 u/µl			2			2			2		
H2O	6.5 →			81.8	81.8	83.8	85 →			69 —	
	Vf = 100 µl										

preheat to 70°C, start by addition of DNA pol
remove 10 µl to 5 µl cycle seq stop mix at: 10, 20, 30 min
well #1 is 23mer unext

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✓
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→ ✓
✓ ← (note Klentaq system relies on Taq storage buffer for glycerol and Tween/NP40 — for THC it is diluted in Taq storage buffer so no supplement is needed for Vent and Deep Vent dilution is in storage buffer (with Triton and 50% glycerol
✓ (1.2 mM $MgOAc$ Cf)
✓ (1.2 mM $MgSO_4$ Cf)
✓ Cf = 5% glycerol
→ ✓ Cf = 2% DMSO
→ ✓
2 } dilute in Vent/Deep Vent storage/dilution buffer (its 0.1% Triton)
2 (dilute in Taq storage buffer)
→ ✓ ∴ Cf = .002% Triton will include 2 µl Taq storage buffer with Triton (similar to 7 µl storage buffer with 0.5% Tween/NP40

3 mM, 0.66 mM/λ 13.5 µl (8.91 pmol)
2.3 mM 16.8 µl (23 pmol)
0.16 mM/λ 13.5 µl
H2O 24.7 µl
55 µl
0.36 pmol primers

** for 32P 23 mer mix → 32P 23 mer of PT plus 16.8 µl cold 5'P 23 mer plus 24.7 µl H2O ∴ Vf = 55 µl and specific activity is reduced ~~4-1×~~ ✓ 2×

* 10× Klentaq is 500 mM Tris HCl pH 9.1 160 mM $(NH_4)_2SO_4$ and NO $MgSO_4$

Cf = 360 n M primers

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